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Endocytosis by Random Initiation and Stabilization of Clathrin-Coated Pits

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Supplemental Figure S1. Calculation of Association Times between LDL and a Forming Clathrin-Coated Pit

To substantiate the experimental times measured for the association of a coated pit with an LDL/LDL receptor complex, we estimated this time by computational and analytical means. MATLAB (Mathworks, Natick, MA) was used to simulate a two-dimensional random of a particle (the LDL/LDL receptor complex) on a grid with a size of $50 \times 50 \mu\text{m}^2$. This field also contained randomly appearing clathrin pits, whose creation and lifetime dynamics matched the experimental observations (three events per 10^8 nm^2 per second, lifetime 20 s). The step size of the simulation assumes a footprint of 15 nm for the interacting parts of the LDL receptor and the clathrin coat. Combined with a stepping time of 1 ms, this results in a two-dimensional diffusion constant for the LDL receptor of $6 \times 10^{-10} \text{ cm}^2/\text{s}$. Simulations were performed for 5000 steps (5 s), and association times of LDL/LDL receptor complexes by a coated pit with were recorded and displayed as an histogram. The distribution can be accurately fit with a monoexponential decay with a time constant of $28 \pm 1 \text{ s}$. An analytical expression for the mean diffusion time τ in two dimensions required to reach a small target of radius a in the middle of a space of radius R ($R \gg a$) is given by $\tau = (R^2/2D) \ln (R/a)$ where D indicates the two-dimensional diffusion constant (Creighton, 1993). The radius R describes the area in which we can find exactly one clathrin pit with which the LDL/LDL receptor complex can associate. Given the experimental observation that three pits are created per 10^8 nm^2 per s and a pit lifetime of 20 s, R equals 730 nm. For $a = 15 \text{ nm}$ and $D = 6 \times 10^{-10} \text{ cm}^2/\text{s}$, this results in an association time of 17.2 s.

Supplemental Movie S1. Dynamic Behavior of Clathrin-Coated Pits and Vesicles Labeled with LCa-YFP

Time-lapse series (120 frames, 10 s intervals acquired at 37°C) obtained from a cell constitutively expressing LCa-YFP using the spinning disk confocal microscope.

Supplemental Movie S2. Dynamic Behavior of Clathrin-Coated Pits and Vesicles Labeled with $\sigma 2$ -EGFP

Time-lapse series (200 frames, 3 s intervals acquired at 37°C) obtained from a cell constitutively expressing $\sigma 2$ -EGFP using the spinning disk confocal microscope.

Supplemental Movie S3. Automatic Selection of Clathrin Clusters Labeled with LCa-YFP by Spot Determination and Particle Tracking

The time-lapse series (100 frames, 3 s intervals acquired at 37°C and subjected to no neighbor deconvolution) corresponds to a confocal optical section obtained from the bottom surface of a BSC1 cell constitutively expressing LCa-YFP (green) and includes the position and shape of the overlapping masks as determined by the automated procedure implemented in SlideBook 4 (blue).

Supplemental Movie S4. Automatic Selection of Clathrin Clusters Labeled with σ 2-EGFP by Spot Determination and Particle Tracking

The time-lapse series (200 frames, 3 s intervals acquired at 37 °C) corresponds to a confocal optical section obtained from the bottom surface of a BSC1 cell constitutively expressing σ 2-EGFP (green) and includes the position and shape of the overlapping masks as determined by the automated procedure implemented in SlideBook 4 (blue).

Supplemental Movie S5. Inhibition of Dil-LDL Entry by Transient Treatment of the Cells with Hypertonic Sucrose

The time-lapse series (60 frames, 10 s intervals acquired at 37 °C) corresponds to a confocal optical section obtained from the top surface of a BSC1 cell constitutively expressing LCa-YFP (green) pretreated for 30 min with hypertonic media (media supplemented with 0.45 M sucrose). Dil-LDL (red) was added 5 min prior to image acquisition. This treatment prevents the dynamic behavior of clathrin and blocks the accumulation of Dil-LDL inside cells. Under this condition, the motion of Dil-LDL on the cell surface is not affected.

Supplemental Movie S6. Cholesterol Depletion Interferes with the Dynamics of Clathrin-Coated Pits and Vesicles

The time-lapse series (60 frames, 10 s intervals acquired at 37 °C) corresponds to a confocal optical section obtained from the bottom surface of a BSC1 cell constitutively expressing LCa-YFP (green) pretreated with ~10 M β -methyl cyclodextrin for 30 min. Transferrin alexa647 was added for another 20 min before acquisition of the movie. The first frame shows absence of the fluorescent signal of transferrin alexa647 (blue) inside cells and its accumulation in the cell surface, consistent with a complete block of its receptor-mediated endocytosis mediated by the clathrin pathway. The LCa-YFP signals (green) are mostly static.

Supplemental Movie S7. Expression of Dynamin 2^{K44A} Interferes with the Dynamics of Clathrin-Coated Pits and Vesicles

The time-lapse series (60 frames, 10 s intervals acquired at 37 °C) corresponds to a confocal optical section obtained from the bottom surface of a BSC1 cell constitutively expressing LCa-YFP (green) and transiently expressing dynamin 2^{K44A}-RFP for 80 hr (red). Transferrin Alexa647 (blue) was added for 20 min before acquisition of the movie. The first frame shows absence of the fluorescent signal of transferrin alexa647 (blue) inside cells and its accumulation in the cell surface, consistent with block of its receptor-mediated endocytosis.

Supplemental Movie S8. Dynamic Behavior of Clathrin and Dynamin

The time-lapse series (30 frames, 6 s intervals acquired at 37°C) corresponds to a confocal optical section obtained from the bottom surface of a COS cell transiently expressing LCa-mRFP (red) and dynamin2-EGFP (green). The outlines illustrate examples of short- and long-lived pits. The long-lived pit displays the characteristic increase in the dynamin signal, followed by the lateral movement of the clathrin vesicle and ending with uncoating.

Supplemental Movie S9. Capture of a Single LDL Particle by a Clathrin-Coated Pit and its Internalization by a Coated Vesicle

Time-lapse series (149 frames obtained with 2.6 s intervals and imaged at 37°C) from a BSC1 cell expressing LCa-YFP (green) and incubated with fluorescently labeled Dil-LDL (red). This example shows the migration and capture of an LDL particle to a newly formed coated pit followed by their coassociation and ending with clathrin uncoating and movement of the LDL particle inside the cell.

Supplemental Movie S10. Capture of a Single Reovirus Particle by a Clathrin-Coated Pit and its Internalization by a Coated Vesicle

Time-lapse series (two consecutive acquisitions of 50 frames obtained with 4 s intervals and imaged at 37°C) from a BSC1 cell expressing LCa-YFP (green) and incubated with Alexa647-labeled reovirus (red). This example shows assembly of a newly formed coated pit under a stationary reovirus particle, followed by acquisition of lateral motion of the virus/clathrin coat complex and ending with clathrin uncoating and further movement of the virus inside the cell. Scale bar, 1 μm .

Supplemental Movie S11. Inhibition of Reovirus Entry by Transient Treatment with Hypertonic Sucrose

Time-lapse series (50 frames, 20 s intervals acquired at 37°C) corresponds to a confocal optical section from the top surface of a BSC1 cell constitutively expressing LCa-YFP (green) pretreated for 30 min with hypertonic media (media supplemented with 0.45 M sucrose). Alexa647 reovirus (red) was added for 20 min prior to acquisition of the movie. Incubation with hypertonic media prevents the dynamic behavior of clathrin and blocks virus uptake (represented here by absence of mobile virus). The highly mobile reovirus particles are those free in the bathing medium. We also used the same blocking conditions but only applied during the infection period carried out using a multiplicity of infection of 0.2–0.5; based on a single cell immunofluorescence-based assay (DeTulleo and Kirchhausen, 1998), we observed a substantial decrease in reovirus infection (reduction of 84%, $n = 288$).

Supplemental Movie S12. Inhibition of Reovirus Entry by Transient Expression of $\Delta 95$ –295 Eps15-EGFP

Time-lapse series (20 frames, 10 s intervals acquired at 37 °C) corresponds to a confocal optical section of two adjacent cells, the one in the left transiently expressing $\Delta 95$ –295 Eps15-EGFP (green). Alexa647 reovirus (red) was added for 75 min prior to acquisition of the movie. Most reovirus particles are immobile and remain on the plasma membrane in the cell (left) expressing $\Delta 95$ –295 Eps15-EGFP. Reovirus internalizes and becomes mobile inside the nonexpressor cell used as a control (right). Using the single cell immunofluorescence-based assay and a multiplicity of infection of 0.2–0.5, we observed a reduction of almost 50% in the frequency of reovirus infection in cells blocked in their clathrin-mediated transferrin uptake by expression of $\Delta 95$ –295Eps15 (n = 192).